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## Optimal Isolation Conditions for Ion-Transport Studies in Pig Aortic Endothelial-Cells

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**W-Pos270** PATCH CLAMP ANATOMY: HIGH VOLTAGE ELECTRON MICROSCOPY OF INVIVO PATCHES. F.Jung, M.J. Song\* and F. Sachs, Biophysics, SUNY, Buffalo, NY and \*Wadsworth Laboratories, NYS Dept. Health, Albany, NY.

Although a great deal of work has been done on the electrophysiology of membrane patches, little is known about the structure of the patch. In order to view the structure of a patch "in-vivo", i.e. in the pipette, we have used high voltage electron microscopy to examine excised patches. Inside-out patches from chick myocytes are formed in a unirepolished borosilicate pipette. The tip is kept under saline and attached to an EM grid using fast cure epoxy. The pipette barrel is broken off leaving about 1mm of tip attached to the grid. This tip may then be fixed and stained. The grid is then either quick frozen in liquid N<sub>2</sub> and freeze dried or critical point dried with actone and CO<sub>2</sub>. In the 1.2MV microscope, the pipette is transparent for about 20 microns from the tip. Useful magnifications up to net 300,000 can be obtained in the tip region. Preliminary results indicate that the inside out patch must be considered as a "bud" containing cytoskeleton and cytoplasm rather than a piece of "pure membrane". It appears quite feasible to use gold labelled antibodies to study the histochemistry of the patch. Studies of outside-out patches are in progress.

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**W-Pos271** IONIC PROPERTIES OF THE SUGAR BEET ROOT VACUOLE MEMBRANE.

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The vacuole is a membrane bound organelle occupying most of the intracellular space of plant cells. Its function is regulatory, sequestering and releasing ions, metabolites, organic acids and water. While it is well known that the regulation is accomplished by the movement of these substances across the vacuolar membrane, no direct evidence has been available until only recently. It has now been shown that the membrane of the barley vacuole has ion channels and an ATPase which pumps H<sup>+</sup> ions into the vacuole (Hedrich et al. FEBS Lett. 204, 228 1986).

We have studied the membrane properties of the sugar beet root vacuole using the patch-clamp technique. This membrane has so far shown two kinds of channels. A predominant large current channel and a less frequent one of about one fifth the conductance. Our report concerns only the large channel observed in whole-cell and outside-out patch configurations and an ATP-activated current in the whole-cell configuration.

The channel opening was very voltage dependent, showing almost no openings for potentials more positive than -20 mV inside to outside. When open, its conductance was 70 pS and 180 pS with symmetrical 50 and 200 mM KCl solutions respectively. The permeability ratios pK/pi for the ions tested were: Na<sup>+</sup>=1, Cl<sup>-</sup>=6, Malate<sup>2-</sup>=5.1, NO<sub>3</sub><sup>-</sup>=3.2, Acetate<sup>-</sup>=2.7.

In the whole cell configuration, applying 5 mM ATP in the bathing medium produced a current of about 60pA which could be sustained for many minutes and disappeared with wash-out of the ATP. In current clamp, the ATP produced a potential shift of up to +55 mV. These effects are believed to be due to the pumping of H<sup>+</sup> ions.

Our present results show that the vacuolar membrane is well adapted to the patch clamp technique and in view of its limited channel diversity, may lend itself very well to the study of transport mechanisms.

**W-Pos272** OPTIMAL ISOLATION CONDITIONS FOR ION TRANSPORT STUDIES IN PIG AORTIC ENDOTHELIAL CELLS. NC Adragna, Wright State Univ., School of Medicine, Dept. Pharm/Tox, Dayton, OH 45435.

Endothelial cells (EC) are highly susceptible to injury as reflected in rapid changes of the normal steady state cation composition. Thus, for ion transport studies it is necessary to assess different methods of isolation and culture conditions to establish their suitability to preserve cell integrity as well as to provide an optimal yield. As a first step in this attempt, the effect of 3 factors was studied on cell count and viability in freshly isolated EC. The method by Gimbrone et al (TCA Manual, 4:813, 1978) was used as a reference. 1). Change in bivalent cations (Ca and Mg) in the isolation medium for collagenase treatment (control, 1mg/ml, 40 min.). Mg alone (0.5mM) gave the highest viability when compared to the control containing the two bivalents ( $\bar{x} \pm SE$ ) (93.8±1.0 vs 74.2 ± 3.2%, respectively, p<0.01). The lowest viability was obtained in the presence of the two bivalent chelators EDTA+EGTA (0.05% each), (53.3±2.6%, p<0.01). In addition, a significantly higher number of cells and protein content per aorta (p<0.05 and p<0.001, respectively) was obtained with Mg alone. In Ca alone (0.9mM), the number of cells/μg protein was higher (p<0.05) than in Mg alone suggesting that cell shrinkage has occurred in the presence of Ca without Mg. These results indicate that Mg but not Ca is necessary during collagenase treatment. 2). Optimal incubation time with 1mg/ml collagenase was determined using 5,15,40 and 60 min incubations. Sixty minutes gave the best yield with no change in viability with respect to the control. 3). Optimal concentration of collagenase was determined using 0.1,0.3,0.5 and 1mg/ml. The highest viability was observed with 0.3mg/ml with no difference in the yield. It is concluded that 0.5mM Mg, 60 min and 0.3mg/ml collagenase provide the best conditions in terms of viability and yield. Supported by WSU Seed grant and Miami Valley Heart Chapter, AHA MV-86-01.